

Chemistry

Larvicidal activity of the Total Alkaloid Fraction of *Prosopis juliflora* (SW) D.C. leaves on *Aedes aegypti* (Linnaeus, 1762) larvae: an experimental and statistical approach

Atividade larvicida da Fração de Alcaloides Totais das folhas de *Prosopis juliflora* (SW) D.C. sobre larvas de *Aedes aegypti* (Linnaeus, 1762): uma abordagem experimental e estatística

Jonatas Tavares da Silva¹ , Maria Danielle Gomes de Lima¹ ,
Renato Augusto da Silva¹ , Plínio Pereira Gomes Junior¹ ,
Leandro Ricardo de Lucena¹ , Thiago Muniz de Souza¹ 

¹ Universidade Federal Rural de Pernambuco, Recife, PE, Brasil

ABSTRACT

Aedes (Stegomyia) aegypti (Linnaeus, 1762) is responsible for several epidemics of dengue, chikungunya, Zika virus, and urban yellow fever, with vector control being the best way to combat the insect. This study aims to evaluate the effect of the total alkaloid fraction extracted from the leaves of *Prosopis juliflora* on *A. aegypti* larvae at the L₃ stage. The total alkaloid fraction was obtained using the methodology described by Tabosa et al., 2000. The bioassays were conducted in a completely randomized design following methodology adapted from the World Health Organization et al., 2005 and Cruz et al, 2019 with seven treatment groups: four test groups corresponding to the alkaloid extract at concentrations of 70, 140, 280, and 560 ppm, a control group (no substance), and two reference standards formulated by the larvicides Pyriproxyfen at 2.0 ppm and NatularTM DT Espinosade[®] at 0.52 ppm. The results were calculated using the Kaplan-Meier test with GraphPad Prism Software 8.0.1 for Windows. It was observed that the plant extracts significantly reduced both the survival probability and the lifespan of *A. aegypti* larvae compared to the control (log-rank test, $\chi^2 = 292.4$; df = 4; p < 0.0001). Severe malnutrition, paralysis, and convulsions were observed prior to death, with the greatest larvicidal activity occurring within the first 60 hours of exposure.

Keywords: *Aedes aegypti*; Piperidine; Dengue; Larvicide; *Prosopis juliflora*

RESUMO

Aedes (Stegomyia) aegypti (Linnaeus, 1762) é responsável por diversas epidemias de Dengue, Chikungunya, Zika vírus e da Febre Amarela Urbana, sendo o controle vetorial a melhor forma de combate ao inseto. Assim, o presente trabalho visa avaliar o efeito da fração de alcaloides totais (FAT) extraídos das folhas da *P. juliflora* sobre larvas do *A. aegypti* no estágio L₃. A fração de alcaloides totais (FAT) foi obtida a partir de metodologia descrita por Tabosa et al., 2000. Os bioensaios foram conduzidos em delineamento inteiramente casualizado (DIC) seguindo metodologia adaptada da World Health Organization et al., 2005 e de Cruz et al., 2019 com sete grupos de tratamento, dos quais: quatro grupos testes correspondem ao extrato de alcaloides nas concentrações de 70, 140, 280 e 560 ppm; um grupo controle (sem substância) e dois padrões de referência formulados pelos larvicidas Pyriproxyfen a 2,0 ppm e NATULAR™ DT ESPINOSADE® a 0,52 ppm. Os resultados foram estimados por meio do teste de Kaplan-Meier usando o programa GraphPadPrism Software 8.0.1 para Windows. Observou-se que os extratos vegetais reduziram significativamente a probabilidade de sobrevivência e o tempo de vida das larvas do *A. aegypti* quando comparada com o controle (teste de log-rank, $X^2= 292.4$; $df= 4$; $p < 0.0001$), sendo observados quadros de desnutrição severa, paralisia e convulsões que precediam a morte, com uma maior atividade larvicida nas primeiras 60 horas de exposição.

Palavras-chave: *Aedes aegypti*, Alcaloides piperidínicos; Dengue; Larvicida; *Prosopis juliflora*

1 INTRODUCTION

In the Americas, the species *Aedes (Stegomyia) aegypti* Linnaeus (1762) is classified as the main urban vector for the transmission of the dengue virus, an infectious disease caused by an arbovirus of the Flavivirus genus, belonging to the *Flaviviridae* family, with four known serotypes worldwide (DENV-1 to DENV-4) Santana et al. (2019); Knakiewicz et al. (2020); Barros et al. (2021). Its clinical manifestations can vary from inapparent and asymptomatic infections to high fever with an abrupt onset, called Dengue Fever or Classic Dengue, severe cases of infection that can progress to hemorrhage (Dengue Hemorrhagic Fever - DHF), shock (Dengue Shock Syndrome - DSS), and death Zhang, Wang (2020). In addition to dengue, the mosquito is also responsible for transmitting the Chikungunya virus, Zika virus, and urban yellow fever Santana et al. (2019); Teixeira et al. (2021).

Arboviruses transmitted by *A. aegypti* have been classified as a serious public health problem worldwide, especially in tropical and subtropical countries where the insect finds favorable conditions for reproduction Martinez et al. (2019). Except

for Chile and Uruguay, the countries associated with Mercosur have the highest incidence of dengue cases in the world, especially Brazil Masciadri (2019). Considering the impossibility of extinguishing the insect due to its high adaptability and wide distribution in urban environments, vector control has been studied and applied by most countries Amlalo et al. (2022).

In agreement with the World Health Organization (WHO), integrated vector management must be based on an efficient, economical, and safe combination of control methods that are reliable, practical, and standardized. This approach should also include simple and effective preventive actions to monitor and combat the mosquito, so that the actions employed can be adopted by the entire population, especially those with low socioeconomic development World Health Organization et al. (2009); World Health Organization (2012).

Nowadays, the main strategies adopted to combat the vector are organized into three basic categories: Physical or mechanical control, biological control, and chemical control, with the latter being the most widely used Zequi et al. (2018). Following the ban on dichlorodiphenyltrichloroethane in most industrialized countries from the 1970s onwards, several classes of molecules emerged as successors to organochlorines and gained great prominence in the late 20th and early 21st centuries. These include compounds belonging to the groups of organophosphates, carbamates, and pyrethroids. Pyrethroids act directly on the central nervous system of mosquitoes but differ in terms of the binding site and the way they act on this nervous system.

However, the constant and indiscriminate use of synthetic chemical substances against *A. aegypti* has led to artificial selection, resulting in the detection of more resistant insect populations. Therefore, strategies for technological innovations to combat the vector have become relevant and necessary Zara et al. (2016); Costa et al. (2020). In this context, plant extracts have gained visibility due to the presence of a wide range of active secondary metabolites that have variable toxic characteristics depending on the evolutionary stage of the insect.

In the larval stage, plant extracts have the potential to act as growth hormone inhibitors, causing physical or neurological deformities that can lead to sterility or inhibit larval feeding, inducing severe malnutrition (starvation). This stage may be the main reason for acute intoxications that lead to the death of larvae from simple contact with the plant extract Silvério et al. (2020); Carvalho, Cruz, and Souza (2023). Additionally, the properties of certain plant compounds, when applied in isolation or in combination with other metabolites, can lead to an increase or decrease in larvicidal activity Serdeiro et al. (2017).

The plant species *Prosopis juliflora* (SW) D.C., known in Brazil as algaroba or algarobeira, belongs to the legume family (*Leguminosae*), subfamily *Mimosoideae*, and is widely distributed in various biomes around the world, with a greater prevalence in arid, semi-arid, tropical, and subtropical areas. It has been intensively investigated for its bacterial activities Satich, Raveesha, Anardhana (1999), fungicidal properties Cunha (2012), antitumor effects Costa; Cavalcante (2018), insecticidal properties Lima et al. (2020), larvicidal effects Azevedo et al. (2021a), (2021b), among others.

This plant species is known for the presence of numerous secondary metabolites throughout the plant, especially flavonoids, tannins, ellagic acid glycosides, and alkaloids. The alkaloids stand out due to the presence of several alkaloids derived from the amino acid L-lysine, which have at least one piperidine ring in the structure of the molecule Oliveira (2018).

Singh and Verna (2012) reported finding a total of thirteen different alkaloids present in algaroba and distributed in distinct parts of the plant, using the Direct Analysis in Real Time Mass Spectrometry (DART-MS) method. Teixeira (2018), on the other hand, analyzed the aqueous leaf extract of *P. juliflora* using the UPLC-QTOF-MS technique, which enabled the identification of six alkaloids already reported for the species in previous studies and four alkaloids that had not been reported for *P. juliflora* until then, but are present in a species from the same family, *Cassia spectabilis* (*Leguminosae*).

Given the great biological potential of the piperidine alkaloids of *P. juliflora* on various classes of insects, this article reports the larvicidal activity of the aqueous extract of the Total Alkaloid Fraction (TAF). The extract was obtained from the leaves of *P. juliflora* on third instar *A. aegypti* larvae under laboratory conditions.

2 MATERIALS AND METHODS

2.1 Egg collection: Setting up the traps

For the collection of population samples of *A. aegypti* eggs, oviposition traps (ovitrap) made from reused polyethylene bottles (PETs) painted black with a capacity of 500 mL were used Brun et al. (2020). Inside, 300 mL of a 0.04% w/v yeast of brewer attractant solution was added, and a pressed wooden paddle (Eucatex type) measuring 3 cm x 12 cm with a rough surface facing upwards was fixed so that the females of the species could lay eggs Zequi et al. (2018); Santana et al. (2019); Lima et al. (2022). The ovitraps were installed in several homes in the city of Princesa Isabel in the state of Paraíba -Brazil, located at the coordinates: latitude 07°44'12" south and longitude 37°59'36" west, with an altitude of 683 meters and at a 420 km distance from the capital João Pessoa, Brazil. Collections were carried out periodically every 5 days, the straws replaced, the traps cleaned, and a new attractant solution was added. The collected material was shade-dried for 48 hours and monitored using a HAIZ USB portable digital microscope (model HZ 1600x, cam 2.0 Mp) to identify and quantify the eggs Gonçalves, Sá et al. (2019); Santana et al. (2019). After analysis, the material was placed in a Styrofoam box before the *in situ* larval rearing stage began.

2.2. Growing *Aedes aegypti* larvae

Aedes aegypti larvae were cultivated in plastic trays containing ultrapure water as a rearing medium, in an air-conditioned room with an average laboratory temperature of 27°C ± 2°C, relative humidity of 60% ± 10% for a 12-hour photoperiod

Thanigaivel et al. (2017); Azevedo et al. (2021a). After hatching, the larvae were periodically fed with dry food for adult cats in mixed flavor from the Purina® Friskies® Megamix brands, previously macerated and sterilized in an autoclave at a temperature of 120°C at 1 atm. The larvae were kept in the trays with water and fed cat food until they reached the third instar (L₃), after which they were randomly selected for the tests Azevedo et al. (2021b).

2.3. Plant raw materials

The aerial parts (leaves) of *Prosopis juliflora* were collected from tree species located near Mata da Pimenteira in the municipality of Serra Talhada, located in the sertão of Pajeú, state of Pernambuco, Brazil. The coordinates are latitude 7°57'34.5" south and longitude 38°17'35.7" west. A sample of the plant species collected was deposited in duplicate in the Herbarium of the Semi-Arid Region of Brazil (HESBRA) at the Federal Rural University of Pernambuco (UFPE), Serra Talhada Academic Unit, with exsiccata number 03512.

The collected plant was dehydrated in an oven at 45°C for 48 hours and then crushed. The plant powder was cold-macerated with 95% ethyl alcohol P.A three times (1:3 m/v) at room temperature for 72 hours, vacuum-filtered, and the aqueous ethanolic extract was concentrated in a rotary evaporator until the solvent was eliminated, obtaining the Crude Ethanolic Extract (CEE) Tabosa et al. (2000).

2.4 Extraction of the Total Alkaloid Fraction (TAF)

The total alkaloid fraction (TAF) from the leaves of *P. juliflora* (algaroba) was obtained by means of an acid-base extraction with an organic solvent in a separating funnel, according to the methodology described by Tabosa et al. (2000), with some minor changes Klein-Júnior & Henriques (2017). First, part of the Crude Ethanolic Extract (CEE) (78.6 g) was solubilized in an aqueous solution of 5% v/v hydrochloric acid (HCl) under mechanical agitation for 2 hours to promote the protonation of

the alkaloids present. Aliquots of the acidified aqueous extract were removed and added to a separating funnel of 500 mL, then subjected to liquid-liquid partitioning with chloroform (3 x 150 mL) under manual stirring for a few minutes to remove lipophilic resins and other apolar interferents. The organic phase was separated from the aqueous phase and discarded. The acidic aqueous phase was alkalized with 3% ammonium hydroxide (NH_4OH) in an ice bath under mechanical stirring for 1 hour until it reached a pH between 10 and 11 to deprotonate the alkaloids present. It was then partitioned with dichloromethane (CH_2Cl_2) (3 x 150 mL) under manual stirring (Cunha; Barbosa Filho, 2014). The basic organic phase was extracted, dehydrated with 5 g of anhydrous sodium sulfate (Na_2SO_4), and left to stand for 2 hours. Afterwards, the solution was filtered through a glass funnel with absorbent cotton and concentrated under vacuum at a controlled temperature of around 45°C in a rotary evaporator, resulting in 1 gram of the final product as a Total Alkaloid Fraction (TAF), which was stored in an amber bottle.

2.5 Bioassays

The Total Alkaloid Fraction (TAF) was solubilized in 30 mL of dimethylsulfoxide (DMSO) (P.A) and then diluted in ultrapure water to concentrations of 70, 140, 280, and 560 ppm at room temperature. The bioassays were conducted in a completely randomized experimental design with seven treatment groups, including: four test groups corresponding to the aqueous alkaloid extract (AAE) of TAF, a control group (ultrapure water without substance and with dimethyl sulfoxide-DMSO), and two reference standard control groups formulated with the commercial larvicide Pyriproxyfen (Ppx) at a concentration of 2 ppm and NATULARTM DT ESPINOSADE® (SpAD) (mixture of Spinosyn A and Spinosyn D) prepared at a concentration of 0.52 ppm. All experiments were conducted in quintuplicate, totaling thirty-five experimental

units. For each experimental unit, ten *A. aegypti* larvae were randomly collected and distributed in disposable polyethylene cups with a capacity of 150 mL containing 100 mL of the solutions, totaling fifty larvae per group. After application, the immature insects were kept on a normal diet (crushed cat food) in an air-conditioned room at 27°C with relative humidity of 60% ± 10% and a 12-hour photoperiod. The bioassays were carried out following a methodology adapted from the World Health Organization et al. (2005) and Cruz et al. (2019).

2.6 Statistical analysis

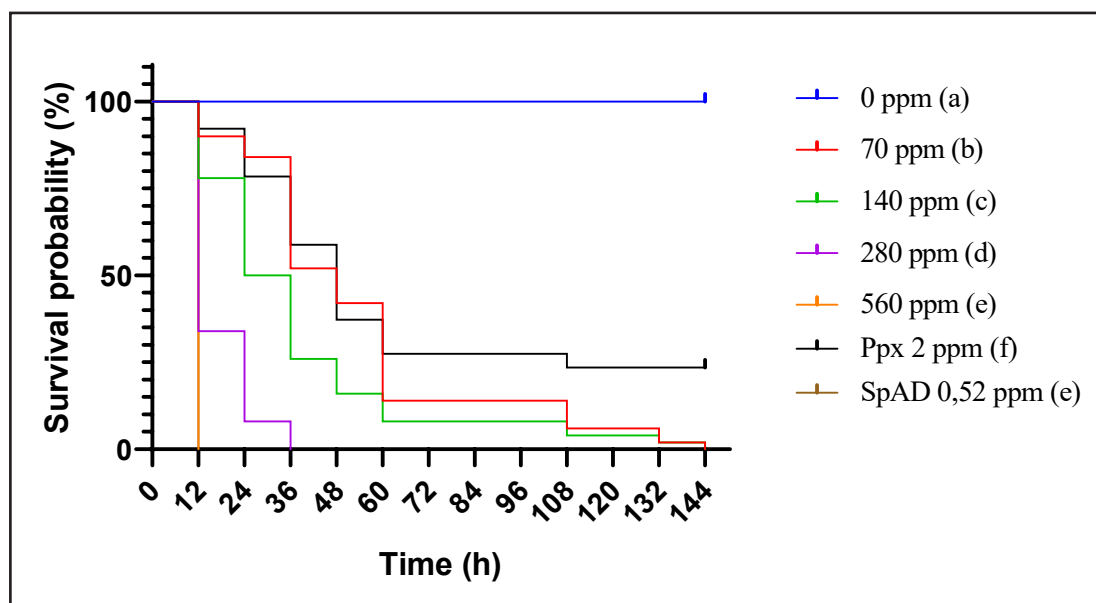
All statistical analyses in this study were carried out using the GraphPad Prism software program (version 8.0.1 for Windows). The data on the survival of *A. aegypti* larvae exposed to the different treatments were subjected to survival analysis. Survival curves and the mean lethal time (LT₅₀) with 95% confidence intervals (CI) were obtained using the Kaplan-Meier estimate. The overall similarity between the curves and the difference in time distributions between the groups (paired comparisons) were calculated using the log-rank statistical test ($p < 0.05$). The results related to the mean survival rates between treatments were subjected to analysis of variance (ANOVA) followed by Tukey test ($p < 0.05$). Probit regression analysis with a 95% confidence interval was used to determine the lethal dose (LD₅₀ and LD₉₀).

3 RESULTS AND DISCUSSION

The effects of the aqueous alkaloid extract (AAE) obtained from the leaves of *P. juliflora*, pyriproxyfen (PPX), and spinosad (SpAD) on third instar *Aedes aegypti* larvae are shown in Figure 1. In the generalized log-rank test, statistically significant differences were found between the survival curves (Pearson's chi-square test ($X^2 = 379.5$; $df = 6$; $p < 0.0001$)), indicating that at least one of the treatments differs significantly from the others at some point in time. The results of the survival analysis showed that the

aqueous alkaloid extract (AAE), at all concentrations evaluated, significantly reduced the probability of survival and the lifetime of the *A. aegypti* larvae compared to the control (log-rank test, $X^2 = 292.4$; $df = 4$; $p < 0.0001$).

Figure 1 – Survival curves of *Aedes aegypti* larvae exposed to different treatments



Source: The Authors (2025). Survival curves calculated using the Kaplan-Meier method for the survival time (in hours) of 3rd instar *Aedes aegypti* larvae exposed to different concentrations (ppm) of the aqueous alkaloid extract of *Prosopis juliflora* leaves (AAE), pyriproxyfen (Ppx) and Espinosade® (SpAD). Treatments coded with the same letter were not significantly different throughout the observation period, according to the log-rank test ($p < 0.05$).

When multiple comparisons were made between the survival curves of the *A. aegypti* larvae exposed to different treatments (Table 1), it was found that there were cases in which the statistical similarity in the pair analyzed varied as a function of time. The results showed that the aqueous alkaloid extract (AAE) at concentrations of 70 and 140 ppm, although showing a statistical difference in the first 60 hours of the experiment ($X^2 = 12.34$; $df = 1$; $p = 0.0004$), no longer differed statistically after this observation period ($X^2 = 0.1383$; $df = 1$; $p < 0.7100$). Treatment with pyriproxyfen (PPX) at a concentration of 2 ppm did not differ statistically from treatment with AAE at a concentration of 70 ppm until 60 hours of exposure ($X^2 = 1.876$; $df = 1$; $p < 0.1708$) but showed a statistical difference after this period ($X^2 = 14.95$; $df = 1$; $p < 0.0001$).

Table 1 – Log-rank test for pairwise comparison of the survival curves of *Aedes aegypti* larvae exposed to the different treatments

Comparison	χ^2	df	p	Comparison	χ^2	df	P <
0 x 70	113.2	1	0.0001	70xSpAD	81.00	1	0.0001
0 x 140	116.8	1	0.0001	140 x 280	30.61	1	0.0001
0 x 280	113.8	1	0.0001	140 x 560	63.30	1	0.0001
0 x 560	99.00	1	0.0001	140xppx	18.24	1	0.0001
70 x 140	12.34	1	0.0004 ⁽¹⁾	140xSpAD	63.30	1	0.0001
70x140	0.1381	1	0.7100 ⁽²⁾	280 x560	20.28	1	0.0001
70 x 280	66.76	1	0.0001	280xPpx	69.08	1	0.0001
70 x 560	81.00	1	0.0001	280xSpAD	20.28	1	0.0001
70xPpx	1.876	1	0.1708 ⁽³⁾	560xPpx	87.79	1	0.0001
70xPpx	14.95	1	0.0001 ⁽⁴⁾	560xSpAD	0.00	1	>0.9999 ⁽⁵⁾

Source: The authors (2025)

(1) The log-rank test showed that the 70 and 140 ppm concentrations of the plant extract were statistically different only in the first 60 hours of the experiment. (2) The 70 and 140 ppm concentrations were no longer statistically different after 60 hours. (3) The 70 ppm EEA treatment and the 2 ppm pyriproxyfen (Ppx) did not differ statistically from each other until 60 hours into the experiment. (4) Treatments differed statistically from each other after 60 hours into the experiment. All other comparisons show statistically significant differences between the curves ($p < 0.0001$) throughout the biological test

It was observed that the pyriproxyfen treatment had a significant reduction in its larvicidal activity, reaching a maximum efficiency of 76% mortality at 108 hours of exposure. The remaining larvae were eliminated after getting into the pupal stage of the insect. In contrast, no pupae were observed in the plant extracts until the end of the bioassays. The AAE at a concentration of 560 ppm was the most lethal of the plant extracts (LT50 of ± 6 hours) and did not differ statistically from the treatment with Spinosad (SpAD), which is currently considered one of the best commercial larvicides against the dengue vector. Both treatments recorded a larval mortality rate of 100% at 12 hours of the experiment. When comparing the extracts alone, it was observed that the statistical significance occurred between the lower and higher concentrations. The aqueous leaf extract of *P. juliflora* showed greater larvicidal activity in the first 60 hours of exposure. The other comparisons showed statistically significant differences throughout the observation period ($p < 0.0001$).

In general, it was observed that, the higher the concentration of the alkaloid leaf extract of *P. juliflora*, the greater the number of failures (deaths) and the shorter the average lifetime of the immature forms of *A. aegypti*. This can be explained by the fact that the increase in the mortality rate is proportionally linked to the increase in the concentration of the plant extract (Table 2) Azevedo et al. (2021a). However, in the lower concentrations of the extract, long exposures to the treatment led to a reduction in larval feeding, due to post-ingestion toxicity and, consequently, severe malnutrition in the immature forms of the vector. It was also observed that the extract affected the locomotor activity of the exposed individuals, slowing them down and causing tremors and convulsions that preceded death.

Similar toxicological effects have been reported for chemical compounds belonging to the organophosphate (OP) class, which act by inhibiting acetylcholinesterase (AChE), an important enzyme in the central nervous system (CNS). The inhibition of AChE causes an accumulation of acetylcholine in the nerve junctions, preventing the interruption of propagation of the electrical impulse, and consequently triggering the process of paralysis, tremors, and convulsions, which can culminate in the death of the insect Braga, Valle (2007).

In addition to its lethal toxic action, the aqueous alkaloid extract was also able to interfere with the evolutionary development of immature forms of *A. aegypti*, prolonging the larval stage (in hours) and delaying or inhibiting the emergence of pupae for at least 144 hours. No previous studies reporting alterations in the evolutionary development of *A. aegypti* larvae caused by the total alkaloid fraction (TAF) extracted from *P. juliflora* leaves were found in literature.

Table 2 – Lethal time to kill 50% (LT₅₀) of *Aedes aegypti* larvae exposed to different concentrations of the aqueous alkaloid extract of *Prosopis juliflora* leaves

Treatment (ppm)	MTF (h)*	CL95%**	LT ₅₀
Control (0)	144 ± 0,0 ^a	144 – 144	-
70	52.56 ± 4.48200 ^b	43.7754 – 61.3446	48
140	37.44 ± 4.16596 ^c	29.2749 – 45.6051	24
280	17.04 ± 1.08908 ^d	14.9054 – 19.1746	12
560	12 ± 0.00 ^e	12 – 12	6

Source: the authors (2025). The results of the log-rank test indicate a significant trend in the reduction of mean survival time when compared to the control ($X^2=292.4$; $df = 4$; $p < 0.0001$). Different letters indicate a significant difference between treatments according to ANOVA followed by Tukey's test ($p < 0.05$). (-) Not determined; * Mean Time to Failure; ** 95% Confidence Interval

This observed secondary effect is considered an important factor in the development of new larvicides, as delaying the insect's life cycle is expected to reduce the number of adults and, consequently, the overall vector population. Therefore, the results presented in this study are promising, which further reinforces the need to study the viability of the piperidine alkaloids present in the plant species as a possible raw material in the formulation of more environmentally friendly larvicides. Cruz et al. (2019) evaluated the action of solasodine, a steroidal alkaloid extracted from *Solanum paludosum*, on immature forms of *A. aegypti*. They found that the alkaloid, isolated at a concentration of 10 µg/mL, interfered with the vector's development, resulting in an increase in its larval, pupal (9.9 ± 2.5 days; $p \geq 0.01$), and emergence (L₃-adult) biological cycles (12.5 ± 2.4 days; $p \geq 0.1$). The results of this work reinforce that alkaloids have considerable biological activity on the vector, indicating that this class of metabolites has enormous potential to be used as promising defensive agents against *A. aegypti*.

Studies carried out with the methanolic extract of *P. juliflora* leaves on third-instar larvae of *Anopheles subpictus*, *Culex quinquefasciatus*, and *A. aegypti* revealed that individuals of the species *An. subpictus* showed the greatest susceptibility to treatments, with a CL₅₀ value of 39.19 ppm, followed by *Cx. quinquefasciatus* and *A. aegypti*, which showed CL₅₀ values of 59.37 ppm and 126.79 ppm, respectively. This

indicates a potential toxic activity of the metabolites present in *P. juliflora* Varuntyagi; Sukumaran; Veer (2015).

The aqueous extract of the powder of dried *P. juliflora* leaves at concentrations of 40 and 50 g/L has shown a larval mortality efficiency of 96.3% and 100% consecutively, after an evaluation period of 168 hours Azevedo et al. (2021b). However, it should be noted that in this study, the fraction of total alkaloids isolated from the leaves at concentrations of 280 and 560 ppm showed a mortality efficiency of 100% after only 36 and 12 hours of exposure respectively. This difference is caused by the aqueous solution of the leaf powder, the low concentration of alkaloids, and the impurities that can interfere with the larvicidal activity. Therefore, it should be emphasized that it is necessary to work with purer alkaloid extracts to obtain better results.

Yadav et al. (2014), while analyzing the larvicidal and oviposition activity of extracts from six plant species in five solvents of different polarity, observed that the methanolic extract of *P. juliflora* was more effective against *A. aegypti* (CL₅₀ of 80.5 ppm), obtaining a larval mortality of 90% at a 400-ppm concentration. It was also a strong inhibitor of oviposition, inhibiting twice as much egg laying (OAI-0.466) at 100 ppm compared to other extracts in the same solvent with larval mortality of 90% at 400 ppm and a CL₅₀ of 80.22 ppm.

In addition, the methanolic extract of the fruit and dried leaves of *P. juliflora* showed an anthelmintic effect *in vitro* on the culture of gastrointestinal nematode larvae in goats, with a reduction of more than 90% in the number of infective larvae for the different types at concentrations of 253.7 to 724.5 mg/L Batatinha et al. (2011). It also exhibited antimalarial action when evaluated *in vitro* with extracts produced by percolation with ethanol from the leaves and pods, causing inhibition of the parasites in the blood at a dose of 2 ppm, similar to the result observed for chloroquine at a dose of 50 ppm Batista et al. (2018). In this study, the values of the lethal doses (in ppm) from the aqueous extract of the alkaloid obtained from the *P. juliflora* leaves required the elimination of 50% and 90% of the larvae (LD₅₀ and LD₉₀), and were then

calculated for 24 and 48 hours using the Probit regression method. The results can be seen in Table 3.

Table 3 – Toxicity by ingestion of the aqueous leaf extract of *Prosopis juliflora* against *Aedes aegypti* larvae

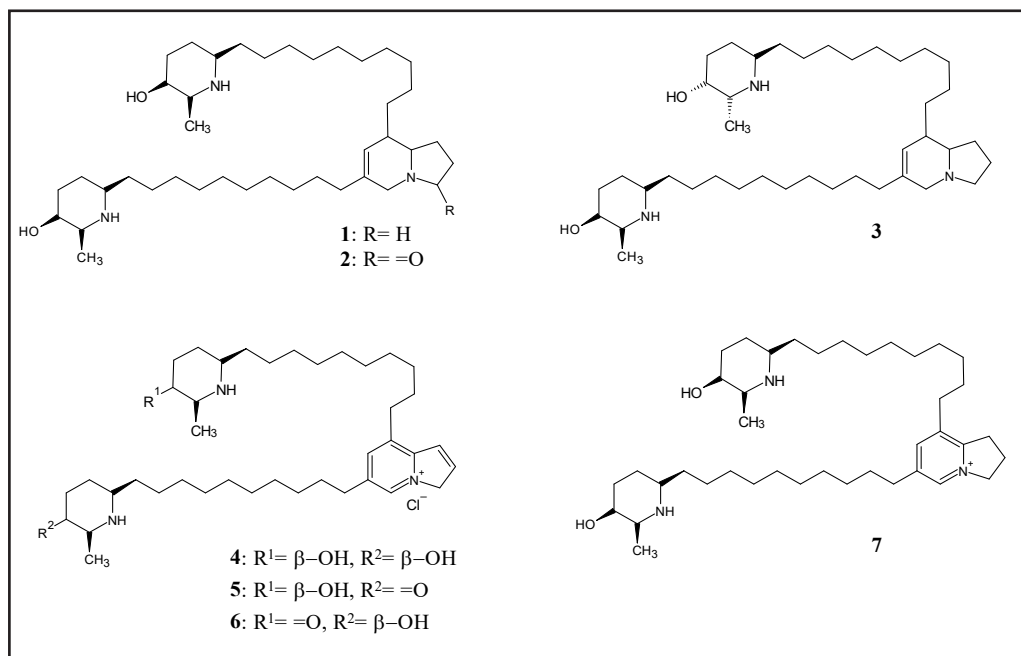
Time of exposure (hours)	Lethal Dose (ppm) ^a		
	LD ₂₀	LD ₅₀	LD ₉₀
24	80.36 [43.00 – 106.39]	170.03 [145.00 – 203.29]	265,52 [22.91 – 333.19]
48	66.27 [38.77 – 87.28]	127.77 [105.08 – 167.42]	221.42 [178.09 – 317.43]

^a Lethal dose of the aqueous alkaloid extract of *P. juliflora* leaves required to kill 20% (LD₂₀), 50% (LD₅₀) and 90% (LD₉₀) of *Aedes aegypti* larvae in 24 and 48 hours of experiment, calculated by Probit regression analysis. Values in curls correspond to a 95% confidence interval

It is worth remarking that the piperidine alkaloids isolated from the leaves Nakano et al. (2004); Singh, Verma (2012) and fruits Tabosa (2000); Batatinha (2011) of *P. juliflora* are classified as the most important secondary metabolites of the plant species, due to their proven toxic activities on various classes of plant and animal organisms.

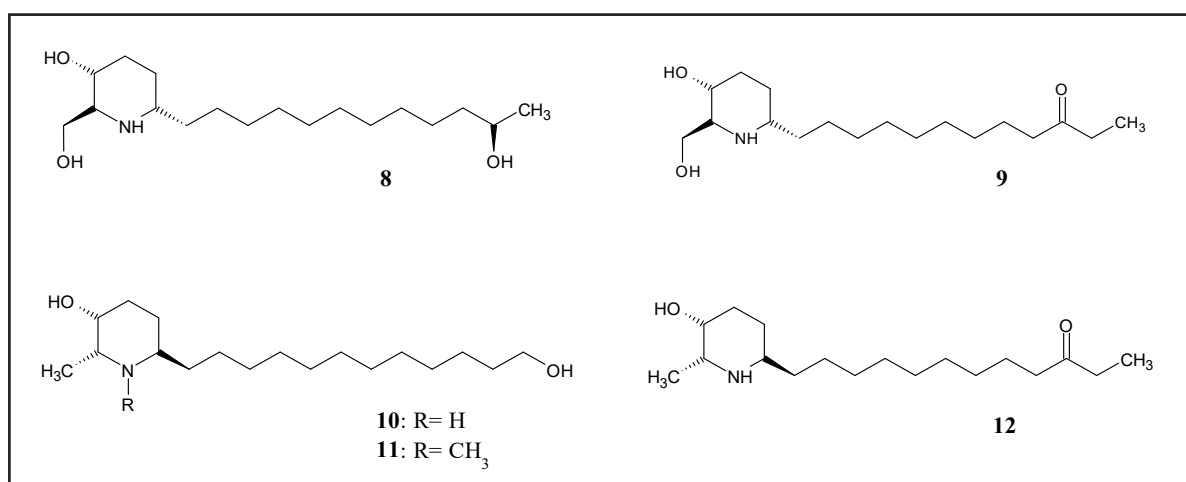
Based on the structures of multiple alkaloids that have already been isolated and characterized in previous research, they can be divided into two distinct groups. The first group has a similar configuration and is made up of alkaloids that have a central indolizidine ring (also derived from L-lysine) and two aliphatic chains that culminate in a piperidine ring (Figure 2). The second group is comprised of alkaloids that have a piperidine ring but no indolizidine ring (Figure 3).

Figure 2 – Chemical structure of the main alkaloids present in *Prosopis juliflora* (group 1): juliprosopine (juliflorine; 1), 3'''-oxo-juliflorine (3'''-oxo-juliprosopine, 2), julifloricine (3), juliprosine (4), 3'-oxo-juliprosine (5), 3-oxo-juliprosine (6) and juliprosinene (7)



Source: The authors, 2025

Figure 3 – Chemical structure of the main alkaloids present in *Prosopis juliflora* (group 2): prosopine (8), prosopinine (9), julifloridine (10), N-methyljulifloridine (11) and prosafrin (12)



Source: The authors, 2025

Oliveira (2018) emphasizes that, the presence of the indole ring located in the center of the structure of some *P. juliflora* piperidine alkaloids is extremely important for the molecule to have a greater toxic effect on the cells of various organisms. Additionally, it is believed that the biological activity of piperidine alkaloids is correlated with the positioning of certain functional chemical groups linked to the carbon atoms of the heterocyclic rings, which make up the structure of the molecule Oliveira (2018); Silva et al. (2018); Teixeira (2018).

Although several studies have shown that aqueous extracts and organic fractions prepared from various parts of *P. juliflora* have the ability to act on different stages of the development of vector Damasceno; Ferrari; Giordani (2017), the exact mechanism by which alkaloids of the plant mediate their larvicidal activity on the immature stages of *A. aegypti* is still poorly understood. However, in order to find new natural cholinesterase inhibitors with the potential to compose drugs used to combat disease of Alzheimer, Choudhary et al. (2005) found that the alkaloid juliprosopine (juliflorine) 1, isolated from *P. juliflora*, showed high in vitro inhibitory activity against *acetylcholinesterase* (AChE) and *butyrylcholinesterase* enzymes (BChE) with non-competitive calcium (Ca^{2+}) channel blockades, depending on the concentration used.

The toxicity of the piperidine alkaloids of *P. juliflora* has also been investigated experimentally in cattle and goats, where neurotoxic damage to the central nervous system (CNS), cachexia, and death were reported, among other symptoms. This intoxication was proven for the first time in Bahia, Brazil, through anatomopathological examinations Figueiredo et al. (1995); Silva et al. (2018); Mendonça et al. (2020). When investigating the implication of programmed cell death (PCD) and autophagy in the cell death mechanism induced by a total extract of alkaloids extracted from *P. juliflora*, as well as a fraction containing mostly the alkaloid juliprosopine 1 in a co-culture model of neural/glial cells, Silva et al. (2017) found that the total extract and the fraction induced a decrease in ATP levels and caused a change in the mitochondrial membrane.

Moreover, they induced caspase-9 activation, nuclear condensation, and neuronal death at 16 hours of exposure, indicating that the neural cell death mechanism induced by the piperidine alkaloids present in *P. juliflora* involves PCD through caspase-9 activation and autophagy. In parallel with neurotoxic activities, juliflorine 1 has been reported to have antibacterial of anti-Alzheimer and antiparasitic activities Henciya et al. (2017). 3-Oxo-juliprosine 6 has been associated with antioxidant and anti-inflammatory activities Sharifi-Rad et al. (2019). Juliprosinene 7 has been found to exhibit antibacterial and antifungal activities Henciya et al. (2017); ABBAS et al. (2022); Martinez et al. (2023). Julifloridine 9 has shown antibacterial, antifungal, and antimalarial activities Henciya et al. (2017) Martinez et al. (2023).

Therefore, based on the observations of the symptoms presented by the *A. aegypti* larvae exposed to the fraction extracts of total alkaloids obtained from the leaves of *P. juliflora*, it is presumed that the piperidine alkaloids present in the extracted fraction must have a neurotoxic mechanism of action on *A. aegypti* larvae, similar to the mechanisms already observed and described in other studies.

4 FINAL CONSIDERATIONS

Considering the need to study more ecological alternatives for controlling *Aedes aegypti*, the aqueous alkaloid extract obtained from the leaves of *Prosopis juliflora* has great potential for use as a botanical larvicide. The results achieved in the study showed that the piperidine alkaloids present in the plant species had lethal toxic activity in vitro against third instar *A. aegypti* larvae, with greater larvicidal activity in the first 60 hours of exposure. Additionally, the extract was able to interfere in the development of the insect, prolonging the larval stage and delaying or inhibiting the incidence of pupation. Further studies are needed to better describe the mechanism of extract of action, as well as the purification of the alkaloids present in the active fraction, tests with the isolated substances, and structural adjustments of the isolated compounds to enhance their biological activities.

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Authorship contributions

1 – Jonatas Tavares da Silva

Master's degree in Chemistry from the Federal Rural University of Pernambuco
<https://orcid.org/0009-0009-2720-3004> • jonatas.silva001.js@gmail.com
Contribution: Methodology, Data curation, Investigation, Writing - first draft

2 – Maria Danielle Gomes de Lima

Bachelor's degree in Chemistry from the Federal Rural University of Pernambuco.
<https://orcid.org/0009-0007-3882-4338> • daniellygoms16@gmail.com
Contribution: Methodology

3 – Renato Augusto da Silva

PhD in Chemistry and Professor at the Federal Rural University of Pernambuco
<https://orcid.org/0000-0002-4064-3954> • renato.augusto@ufrpe.br
Contribution : Investigation, Administration project

4 – Plínio Pereira Gomes Junior

PhD in Genetics at the Federal Rural University of Pernambuco
<https://orcid.org/0000-0002-7318-5793> • plinio.gomesjr@ufrpe.br
Contribution: Conceptualization, Validation, Investigation, Data curation, Writing - review & editing, Supervision, Administration project

5 –Leandro Ricardo de Lucena

PhD in Biometrics and Applied Statistics from the Federal Rural University of Pernambuco
<https://orcid.org/0000-0001-6985-7668> • leandroricardo_est@yahoo.com.br
Contribution: Conceptualization, Validation, Data curation, Writing - review & editing, Supervision

6 – Thiago Muniz de Souza

PhD in Chemistry at the Federal Rural University of Pernambuco
<https://orcid.org/0000-0002-3950-4957> e-mail: thiago.munizs@ufrpe.br
Contribution: Conceptualization, Formal analysis, Investigation, Data curation, Writing - Review & editing, Data visualization, Supervision, Administration project

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